

Attorney Docket No. P64765US1  
Application No. 09/779,461

**Amendments to the claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of claims:**

Claims 1-56 (canceled)

57. (New) A method for characterizing samples having fluorescent particles, comprising the steps of:

- a) monitoring intensity fluctuations of fluorescence emitted by the particles in at least one measurement volume by detecting sequences of photon counts by at least one photon detector,
- b) determining from the sequences of photon counts intermediate statistical data comprising at least two probability functions,  $\hat{P}_1(n_1), \hat{P}_2(n_2), \dots$ , of the number of photon counts,  $n_1, n_2, \dots$ , detected in different sets of counting time intervals,
- c) determining from said intermediate statistical data a distribution of particles as a function of at least two arguments, wherein one argument is a specific brightness of the particles, or a measure thereof, and another argument is a diffusion coefficient of the particles, or a measure thereof,

wherein said distribution function of particles is determined by fitting the experimentally determined probability functions  $\hat{P}_1(n_1), \hat{P}_2(n_2), \dots$  by corresponding theoretical probability

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functions  $P_1(n_1), P_2(n_2), \dots$

and

wherein said theoretical probability distributions  $P_1(n_1), P_2(n_2), \dots$  are calculated as functions of apparent concentrations and apparent brightness which depend on the widths of the counting time intervals in the different sets.

58. (New) The method according to claim 57 wherein, in calculations of the theoretical distributions  $P_1(n_1), P_2(n_2), \dots$ , an optical spatial brightness function  $B(r)$  is accounted for by a separately determined relationship between brightness  $B$  and volume elements  $dV$ .
59. (New) The method according to claim 58, wherein the relationship between the spatial brightness  $B$  and volume elements  $dV$  is expressed through a variable  $x = \ln(B_0/B)$  by a relationship  $\frac{dV}{dx} = A_0(1 + a_1x + a_2x^2)x^{a_3}$ , where  $B_0$  is maximum brightness and  $A_0, a_1, a_2$  and  $a_3$  are empirical parameters of the spatial brightness function.
60. (New) The method according to claim 57 wherein each set of counting time intervals consists of intervals of equal width while different probability functions  $\hat{P}_1(n_1), \hat{P}_2(n_2), \dots$

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correspond to counting time intervals of different widths  $T_1, T_2, \dots$ .

61. (New) The method according to claim 60, wherein the apparent concentration is calculated as

$$c_{app}(T) = \frac{c_{app}(0)}{\Gamma(T)},$$

the apparent brightness is calculated as

$$q_{app}(T) = q_{app}(0)\Gamma(T),$$

and  $\Gamma(T)$  is calculated as

$$\Gamma(T) = \frac{1}{c_{app}(0)q_{app}^2(0)T^2} \int_0^T dt_1 \int_0^T dt_2 G(t_2 - t_1),$$

where  $G(t)$  denotes autocorrelation function of fluorescence intensity and  $T$  denotes the width of the counting time interval.

62. (New) The method according claim 57 wherein the counting time intervals in each set are consecutive in time.

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63. (New) The method according to claim 57 wherein, counting time intervals in each set overlap.

64. (New) The method according to claim 57 wherein said intermediate statistical data are processed applying inverse transformation with regularization and/or constraints.

65. (New) The method according to claim 57 wherein the theoretical distributions  $P_1(n_1), P_2(n_2), \dots$  are calculated through their generating functions

$$G_{P(n)}(\xi) = \sum_n \xi^n P(n).$$

66. (New) The method according to claim 57 wherein said distribution function of particles is determined by fitting the experimentally determined probability functions  $\hat{P}_1(n_1), \hat{P}_2(n_2), \dots$  by corresponding theoretical probability functions  $P_1(n_1), P_2(n_2), \dots$ .

67. (New) The method according to claim 63 wherein the theoretical probability functions

$P_1(n_1), P_2(n_2), \dots$  are calculated through their generating functions  $G_{P(n)}(\xi) = \sum_n \xi^n P(n)$ .

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68. (New) The method according to claim 62, wherein the generating function is calculated using the expression  $G(\xi) = \exp[\int dq c(q) \int d^3r (e^{(\xi-1)qTB(r)} - 1)]$ , where  $c(q)$  is the apparent density of particles with specific brightness  $q$ ,  $T$  is the length of the counting time interval, and  $B(r)$  is the spatial brightness profile as a function of coordinates.
69. (New) The method according to claim 63, wherein said generating functions are calculated using the formula  $G_{p(n)}(\xi) = \exp[\sum_i c_i \int (e^{(\xi-1)q_i B(r)T} - 1) dV]$  in which  $c$  is an apparent concentration and  $q$  is an apparent brightness which both depend on the width of the counting time interval  $T$ .
70. (New) The method according to claim 57 wherein concentrations of particles are selected to be approximately one or less molecules per measurement volume.
71. (New) The method according to claim 57 wherein said photon detector is either an avalanche photodiode or a photomultiplier.

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72. (New) The method according to claim 57 wherein at least two photon detectors are used monitoring fluorescence of different wavelengths or polarization.
73. (New) The method according to claim 57 wherein said fluorescent particles are characterized by applying an homogeneous fluorescence assay.
74. (New) The method according to claim 57, wherein the intensity fluctuation of fluorescence emitted by particles in at least one measurement volume are monitored by detecting sequences of photon counts using at least one photon detector, which photon detector is part of a confocal apparatus.
75. (New) The method according to claim 74, wherein said confocal apparatus comprises:
- a) a radiation source (12) for providing excitation radiation (14),
  - b) an objective (22) for focusing the excitation radiation (14) into a measurement volume (26),
  - c) a detector (42) for detecting emission radiation (30) that stems from the measurement volume (26), and
  - d) an opaque means (44) positioned in the pathway (32) of the emission radiation (30) or excitation radiation (14) for erasing the central part of the emission radiation (30) or excitation radiation (14).